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NOB suppression in pilot-scale mainstream nitritation-denitritation system coupled with MBR for municipal wastewater treatment

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HIGHLIGHTS

- Pilot scale mainstream nitritation-denitritation coupled with MBR was achieved.
- Integrated control of DO, SRT and sludge return ratio was crucial.
- Feasible solutions for the overgrowth of NOB in MBR was shown.
- Both aeration-associated energy and external organic carbon were saved.
- Microbial analysis clearly showed that NOB was suppressed.

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ABSTRACT

The high energy consumption associated with biological treatment of municipal wastewater is posing a serious impact and challenge on the current global wastewater industry and is also inevitably linked to the issue of global climate change. To tackle such an emerging situation, this study aimed to develop strategies to effectively suppress nitrite oxidizing bacteria (NOB) in pilot-scale mainstream nitritation-denitritation system coupled with MBR for municipal wastewater treatment. The results showed that stable nitrite shunt was achieved, while more than 90% of COD and NH_4^+-N removal were obtained via nitritation-denitritation in the pilot plant fed with real municipal wastewater. Through adjusting aeration intensity in MBR in combination with the integrated control of dissolved oxygen (DO), sludge retention time (SRT) and sludge return ratio, NOB was successfully suppressed with a nitrite accumulation rate (NAR) of more than 80%.

1. Introduction

The conventional nitrification-denitrification process has been globally applied for removing ammonia from municipal wastewater, which has the drawbacks of relatively high aeration-associated energy demand and addition of external organic carbon source for wastewater with low C/N ratio. To tackle this challenge, nitritation-denitritation has been explored as an alternative solution, in which ammonia is oxidized to nitrite instead of nitrate, and this may theoretically lead to 25% of saving in the aeration-associated energy consumption. Moreover, denitritation of nitrite to nitrogen gas by heterotrophic denitrifiers could also reduce the demand on external organic carbon by 40% compared to full denitrification (Regmi et al., 2014). However, the mainstream nitritation-denitritation for low strength municipal wastewater treatment is still under development due to the challenges in maintaining a stable microbial community that favours the retention of ammonia oxidizing bacteria (AOB) against nitrite oxidizing bacteria (NOB) (Xu et al., 2017). The free ammonia (FA) and free nitrous acid (FNA) concentrations in low strength municipal wastewater cannot reach levels that are inhibitory to NOB.

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Moreover, the control of dissolved oxygen (DO) and sludge retention time (SRT) may be difficult toward suppression of NOB due to low ammonia concentration (Hulle et al., 2010; Vadivelu et al., 2006). In order to achieve stable nitritation-denitritation, it had been reported in a laboratory-scale study that suppression of NOB could be obtained by intermittent aeration and control of SRT (Regmi et al., 2015). Another study found that low DO condition of 0.3–0.5 mg/L could be favourable for nitritation-denitritation in a laboratory reactor treating domestic wastewater (Zeng et al., 2010). Furthermore, it was shown by Xu et al. that rapid anoxic-oxic shift was critical for effectively repressing NOB activity towards achieving a stable nitrite shunt process (Xu et al., 2017). Some researchers also found that multiple step-feeding strategy was helpful for improving nutrient removal efficiency and could be beneficial for nitritation-denitritation process (Gu et al., 2017; Lemaire et al., 2008; Ge et al., 2014). However, a holistic operation strategy for stable mainstream nitritation-denitritation for the treatment of low strength municipal wastewater has not yet been demonstrated in large scale reactor system.

In addition, membrane bioreactor (MBR), a combination of biological degradation and membrane filtration, has gained increasing popularity in wastewater treatment worldwide due to the advantages of smaller footprint and less sludge production (Cai et al., 2016). Ideally, a mainstream nitritation-denitritation system coupled with MBR would offer a solution for treating municipal wastewater with the aim to produce high-quality permeate at low energy cost. However, some potential challenges may exist for applying mainstream nitritation-denitritation system coupled with MBR. In case of available nitrite, the aerobic condition in MBR may provide a favourable environment for the growth of NOB, which could undermine the mainstream nitritation-denitritation. Especially, it may be highly difficult to suppress the NOB activity in this proposed combined system compared with the traditional nitritation-denitritation process. So far, little information is available for such combined system at large pilot-scale and feasible solutions for the growth of NOB in MBR. In addition, there have been some researches focused on the microbial analysis in the nitritation-denitritation process. It was found that AOB increased from 7.15 x 10^6 to 8.99 x 10^6 copies/g, while NOB decreased approximately threefold in a intermittently aerated system (Hou et al., 2017). In another study, it was reported that AOB population increased 5.9 times and nitrate reductase gene narG was found increasing by 3.4 times in a sequencing batch reactor (Erdirencelebi and Koyuncu, 2017). However, the information of key functional microbial communities for nitrogen removal in this proposed combined system is still lacking.

Given such situation, this study aimed to design and optimize a pilot-scale step-feed activated sludge system coupled with MBR at the treatment capacity of 30 m^3/day towards the stable mainstream nitritation-denitritation. In addition to the analysis of the process performance in terms of COD and nitrogen removal, the abundances of 16S rRNA gene of key functional species for nitrogen removal were also investigated. Moreover, the practical solutions for suppressing the growth of NOB in MBR during the pilot experiment were explored.

2. Materials and methods

2.1. Experimental setup

The pilot-scale step-feed activated sludge system in a local Water Reclamation Plant consisted of a holding tank (2.2 m × 1.0 m ×2.4 m), a biological tank (4.0 m × 1.2 m x 2.0 m) and a MBR tank (1.4 m × 1.2 m x 1.5 m). The biological tank comprised six basins (one standby), each of which had a pair of the alternating anoxic and oxic chambers as schematically illustrated in Fig. 1. In this study, the effluent from a primary settling tank (PST) in the local Water Reclamation Plant was used as the influent wastewater which was fed evenly into five anoxic zones at a flow rate of 0.2Q for each. The effluent from the biological tank was fed into the MBR. The settled sludge was returned back to the first anoxic chamber at a pre-set ratio (sludge return ratio), while SRT was controlled through adjusting the discharging of the wasted activated sludge. A blower was connected to all the oxic chambers with the control valves by which the aeration intensity in each oxic chamber could be adjusted towards a desirable DO level. The treatment capacity of the pilot plant was designed to be 30 m^3/d with a hydraulic retention time (HRT) of 6.4 h and a SRT of 3–10 days. NH_4^+-N was measured with the Hach Nessler reagent set according to USEPA Nessler method (AWWA, 1998). Mixed liquor volatile suspended solids (MLVSS), NO_2^--N, NO_3^--N and COD concentrations were all determined according to standard methods (Eaton et al., 1998). In addition, the COD and ammonia nitrogen concentrations of the influent were around 200 mg/L and 40 mg/L respectively while the nitrite and nitrate concentrations of the influent were under detection limits. The sludge seeded to the pilot study was taken from the returned sludge of MBR tank in the local Water Reclamation Plant (Singapore) and the MLVSS of the seed sludge was around 2500 mg/L.

There are four phases in this pilot-scale experiment. Different operation conditions (i.e. DO, SRT and sludge return ratio) were studied and the optimal combined control conditions were determined for nitritation-denitritation in Phase I. Although the optimal combined control conditions were continually applied, the achieved nitritation-denitritation was lost due to the large fluctuation of influent ammonia concentration in Phase II. After the influent ammonia level was stable, the optimal combined control conditions used in Phase I still could not get nitritation-denitritation back due to the growth of NOB in MBR. Therefore, a re-designed operation strategy was applied for NOB suppression during Phase III. Under the adjustment of aeration intensity in MBR in combination with the integrated control of DO, SRT and sludge return ratio, a stable nitrite shunt was successfully restored in Phase IV.

2.2. Microbial analysis

Sludge samples were taken from the anoxic and oxic chambers of the biological tank of the pilot plant at different time intervals (i.e. day 152, 202, 243 and 271). DNA of the sludge was extracted using a Fast DNA spin kit (MP Biomedicals, LLC). Afterwards, the abundance of 16S rRNA genes of AOB, *Nitrobacter spp., Nitrospira spp.* and denitrifiers were determined by quantitative polymerase chain reaction (q-PCR) using SybrGreen assays with corresponding primers (Araki et al., 2004; Dionisi et al., 2002; Degrange and Bardin, 1995; Throbäck et al., 2004) listed in Table 1.

2.3. Batch experiments

The aim of laboratory batch experiments was to verify the suppression of NOB activity achieved in the pilot plant. Sludge samples were taken from the oxic chambers of the biological tank in the pilot plant on day 255, which was during the stable nitritation-denitritation period in Phase IV. The collected sludge samples were aerated for 2 h to remove the residual COD before the batch experiments. The batch experiments were conducted within 24 h after the sludge samples were taken from the pilot plant. The municipal wastewater used in the pilot plant was fed into the batch reactors. The ammonia oxidation experiments were conducted for 2 h at the DO greater than 2 mg/L, and samples were taken every 30 min and filtered immediately through 0.45 μm filters for

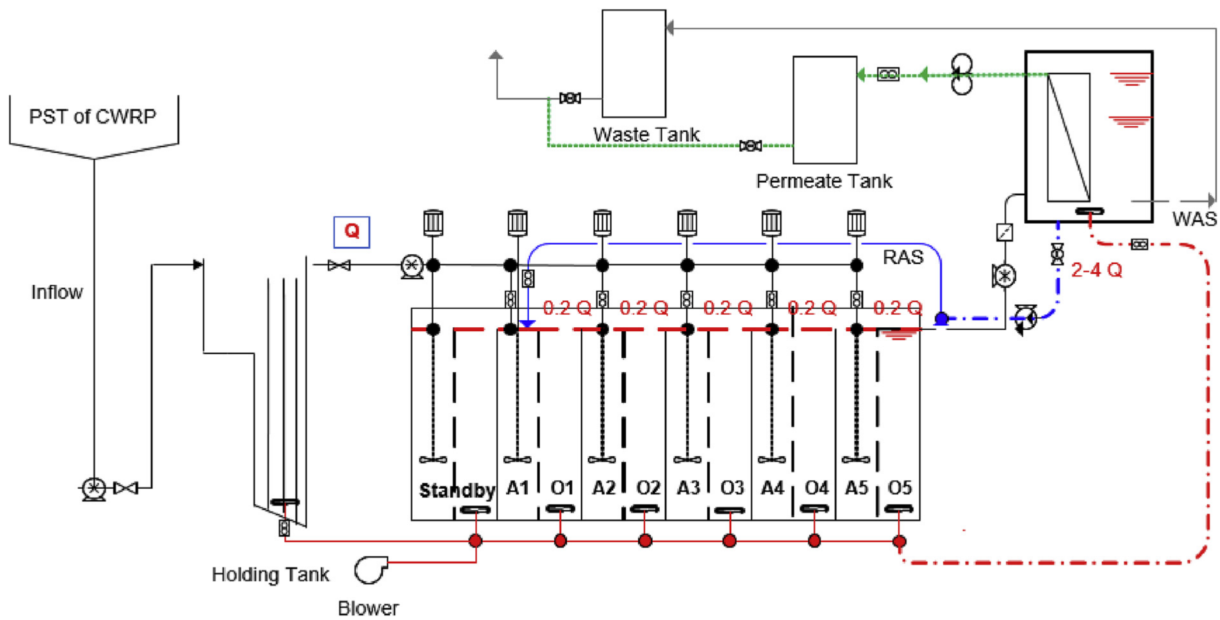

Fig. 1. Schematic diagram of the pilot plant. A1 – A5: Anoxic chambers; O1 – O5: Oxic chambers; RAS: returned activated sludge; WAS: wasted activated sludge.

Table 1
Primers used for q-PCR analysis.

Primers	Gene	Target	Sequence (5′-3′)	Annealing temp. (°C)
CTO189f[a]	16S rRNA	β-Subdivision	CTAGCYTTGTAGTTTCAAACGC	57
CTO654r		AOB		
NSR1113f	16S rRNA	Nitrospira	CCTGCTTTCAGTTGCTACCG	65
NSR1264r			GTTTGCAGCGCTTTGTACCG	
FGPS872	16S rRNA	Nitrobacter	CTAAAACTCAAAGGAATTGA	50
FGPS1269			TTTTTTGAGATTTGCTAG	
nosZF	nosZ	Denitrifiers	CGYTGTTCMTCGACAGCCAG	53
nosZ1622R			CGSACCTTSTTGCCSTYGCG	

[a] Mix of CTO189fA-B (GGAGRAAAGCAGGGGATCG) and CTO189fC (GGAGGAAAGTAGGGGATCG) at ratio of 2:1.

determination of ammonium nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N) and nitrate nitrogen (NO_3^--N) concentrations. The respective AOB and NOB activities were quantified by the NOx^--N and NO_3^--N production rates.

3. Results and discussion

3.1. Combined control of operation parameters for NOB suppression

As introduced above, the whole operation period of the pilot plant was divided into four phases. In Phase I (Day 1 to Day 89), the combined control of different operation parameters was studied for achieving stable nitritation-denitritation. Specifically, with the decrease of DO from 1.7 to 0.5 mg/L in the oxic chambers of biological tank, a slight increase of the nitrite accumulation rate (NAR) was observed at the sludge return ratio and SRT of 1.5Q and 8 days (Fig. 2 (a)). Afterwards, different sludge return ratios were explored at the DO of 0.5 mg/L and SRT of 8 days and 33.8% of NAR was obtained at the sludge return ratio of 2.5Q (Fig. 2 (b)). Besides, under the optimized conditions for DO (i.e. 0.5 mg/L) and sludge return ratio (i.e. 2.5Q), the effect of SRT on nitrite shunt was further evaluated. With the reduction of SRT from 10 to 4 days, a significant increase of NAR was observed (Fig. 2 (c)). Based on these experiment, the optimal operation parameters for the pilot plant were determined to be DO of 0.5 mg/L, SRT of 4 days and sludge return ratio of 2.5Q for achieving stable nitritation-denitritation. After the operation of the pilot plant had been adjusted to these conditions, the nitrite accumulation rate (NAR) in the oxic chambers of the biological tank was gradually stabilized at about 90%, which also confirmed that the activity of NOB was successfully suppressed and a stable nitrite shunt was established. Probably this is the first pilot study demonstrating stable mainstream nitritation-denitritation coupled with MBR for low strength municipal wastewater treatment.

DO and SRT have been considered as two crucial factors for obtaining the nitrite shunt. However, there are still controversies existed about the levels of DO and SRT for benefiting the nitritation-denitritation process (Guisasola et al., 2005; Wu et al., 2016; Aslan and Simsek, 2017; Regmi et al., 2015). The applied DO and SRT levels that are favourable for the nitrite shunt in this study are in agreement with the results of Zeng et al. and Wu et al. 's studies (Zeng et al., 2010; Wu et al., 2016). More importantly, a recent study demonstrated that rapid switch of anoxic and oxic conditions was crucial for establishing a stable nitritation (Xu et al., 2017). This finding indeed was incorporated into the pilot plant operation by adjusting sludge return ratio in the step-feed biological system. Overall, there is still a lack of combined control strategy towards stable nitrite shunt. The results of this study showed that control of single parameter would not lead to a stable nitritation-denitritation, which proved that the combined control strategy of DO (i.e. 0.5 mg/L), SRT (i.e. 4 days) and sludge return ratio (i.e. 2.5Q) was required. In addition, the overgrowth of NOB was not observed,

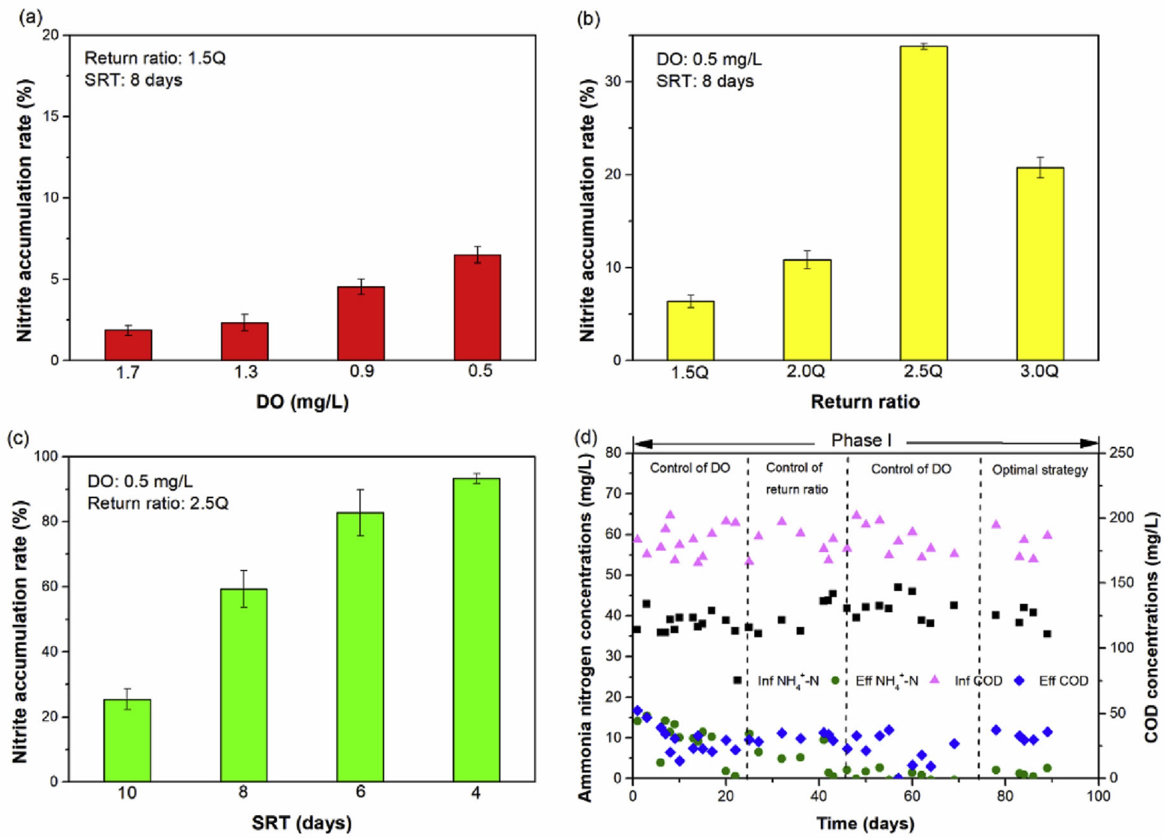

Fig. 2. Optimization of DO (a), return ratio (b) and SRT (c) together with the system performance under the optimal combined conditions (d) in Phase I operation of the pilot plant.

i.e. no significant nitrite oxidation occurred in MBR during Phase I.

As can be seen in Fig. 2 (d), except for the startup period in Phase I, ammonia nitrogen levels of MBR permeate were controlled within 7 mg/L while COD concentrations were maintained below 25 mg/L. As a result, more than 90% of COD and NH_4^+-N removal efficiencies were both achieved in the pilot plant on average. In detail, 89.3% of COD was removed together with 87.3% of NH_4^+-N removed via nitritation-denitritation in the biological tank. Moreover, the MBR could help to further remove COD and NH_4^+-N (Fig. S1 in Supplementary Materials) and the turbidity of the MBR permeate was found to be 0.3 NTU. These indicated that the quality of the MBR permeate was sufficiently good and can meet the effluent discharge standard in terms of ammonia nitrogen, COD and turbidity.

3.2. Suppression of NOB activity in MBR

In Phase II (Day 90 to Day 166), the pilot plant operation was seriously disturbed by a large fluctuation of influent ammonia concentration from 47 to 20 mg/L (Fig. 3(a)), which led to a significant decrease of nitrite concentration from 3 mg/L to below 0.5 mg/L. Meanwhile, the nitrate level was found to increase from below 1 mg/L to more than 10 mg/L (Fig. 3 (b)). These results clearly showed that the growth of NOB started to be out of control in Phase II. As the result, the NAR in the oxic chambers of the biological tank was sharply reduced to less than 1%, indicating that the established stable nitritation-denitritation in Phase I was lost due to the instability of the influent ammonia concentration (Fig. 3 (c)). It should be pointed out that the mainstream nitritation-denitritation coupled with MBR indeed posed a very serious challenge on suppression of NOB versus AOB due to the substantial growth of NOB in MBR, which was also the reason that the optimal combined operation strategy as identified in Phase I could not help to restore nitrite shunt even after the influent ammonia level became stable. In fact, little information is currently available with regard to the mainstream nitritation-denitritation coupled with MBR in the literature.

After carefully analyzing the situation, a new strategy of controlling aeration intensity in MBR combined with the holistic adjustment of DO, SRT and sludge return ratio was further developed and validated in Phase III (Day 167 to Day 219) and Phase IV (Day 220 to Day 303), i.e. the DO concentration in the MBR was reduced from 2.5 mg/L to 2.0 mg/L in Phase III, and finally fixed at 1.5 mg/L in Phase IV. As the result, the nitrite and nitrate concentrations in MBR permeate were increased and reduced at 3 mg/L and 1.5 mg/L respectively (Fig. 3 (b)). Meanwhile, the NAR in the oxic chambers of the biological tank was found to increase in Phase III and stabilized around 80% in Phase IV (Fig. 3 (c)). These clearly indicated that NOB was successfully suppressed by applying the new strategy in the system and a stable nitritation-denitritation was restored and remained stable for almost four months. Moreover, as can be seen in Fig. 3 (a), the quality of the MBR permeate in Phases III and IV was as good as in Phase I and eventually could be used as a feed water for further production of high-grade water (Lee and Tan, 2016). It should be also noted that no external organic source was added to the pilot plant along the entire operation period, while 25% of saving in the aeration-associated energy demand could be achievable in the nitritation-denitritation compared to full nitrification-denitrification.

Overall, a novel pilot mainstream nitritaion-denitritation

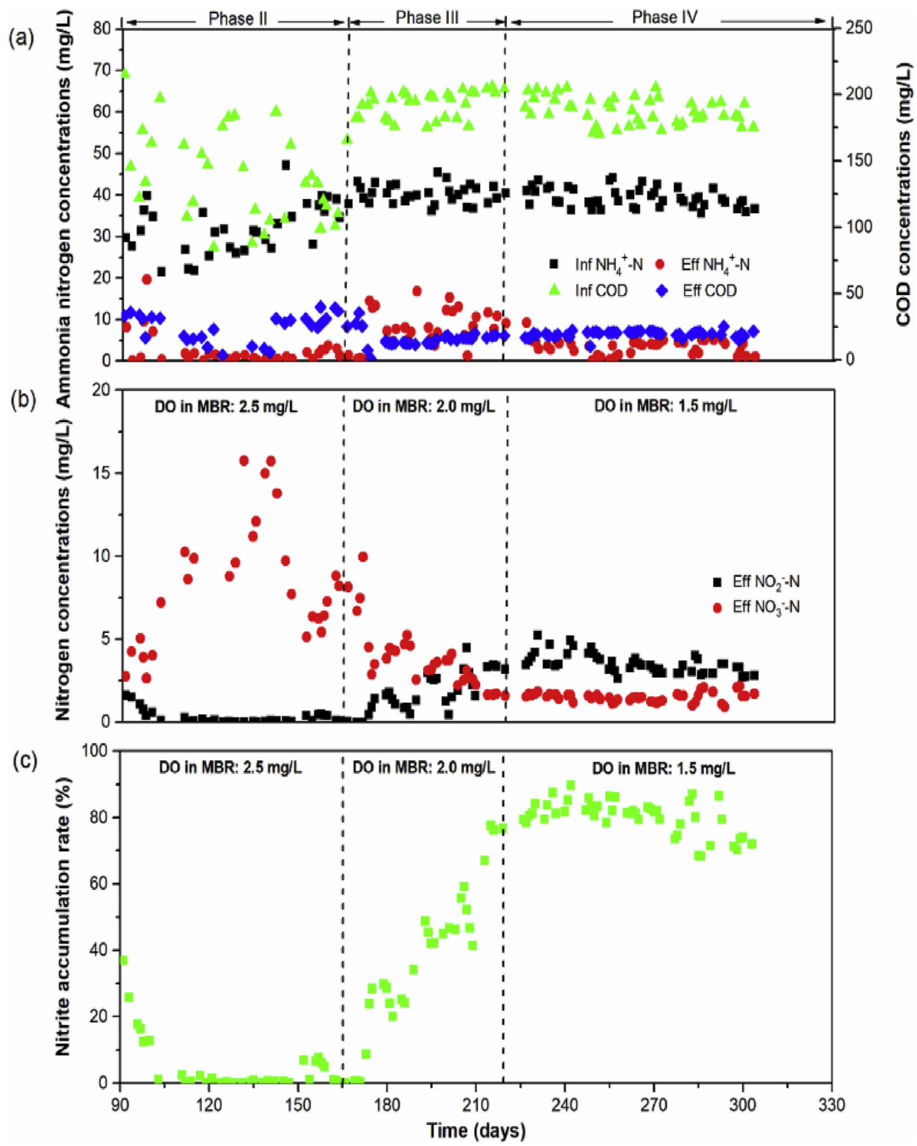

Fig. 3. Concentration profiles of ammonia nitrogen and COD (a) and nitrogen compounds (b) in the pilot plant together with nitrite accumulation rate in oxic chamber of biological tank (c) during Phases II, III and IV.

coupled with MBR was successfully applied for treating low strength municipal wastewater in this study. Different from the previous studies (Sliekers et al., 2005; Wu et al., 2016), a combined control strategy of DO, SRT and sludge return ratio was developed for achieving a stable nitrite shunt. Moreover, it was demonstrated that NOB activity in MBR had a profound impact on nitritation in the upstream biological tank, and it could be suppressed by controlling the aeration intensity in MBR. This in turn may offer guidance for future large-scale municipal used water treatment at low energy cost. Although the mainstream deammonification has been considered as a promising alternative for cost- and energy-effective removal of ammonia from municipal used water, it still faces many operational challenges, e.g. a very narrow operation window for sustaining anammox activity, relatively strict requirement for influent COD/N, difficulty to retain sufficient amount of anammox bacteria (Xu et al., 2015; Strous et al., 1998; Laureni et al., 2016). Therefore, in consideration of the overall benefit/cost and process feasibility/stability, nitrogen removal via nitritation-denitritation could offer a more feasible and practical option for easier and more stable process operation in treating low strength municipal used water.

For detail information of ammonia nitrogen removal, the nitrogen profiles in each chamber of biological tank and MBR on day 242 (i.e. Phase IV) were shown in Table 2. In the oxic chambers, most of ammonium was quickly converted to nitrite and nearly no nitrate was observed (i.e. less than 0.3 mg/L), which led to an average NAR of 89.7%. These results clearly suggested that the NOB activity was significantly suppressed and a stable nitritation-denitritation was obtained. Moreover, by applying multiple step-feed strategy, both nitrite and nitrate concentrations were below 0.3 mg/L, indicating that the excellent denitritation was achieved in the anoxic chambers due to the sufficient COD in the step-fed influent. Due to high efficiency of denitritation, produced nitrite could be quickly denitrified in the anoxic zones. As the result, no enough nitrite was left for the growth of NOB in the subsequent oxic chambers. Consequently, NOB was repressed and gradually washed out under the conditions studied. Although multiple step-feed and alternating anoxic/oxic conditions both in temporal and

Table 2
Nitrogen and COD removal in the pilot plant (mg/L).

Chambers of the system	NH_4^+-N	NO_2^--N	NO_3^--N	COD
Flow to A1[a]	3.32 ± 0.05	0.78 ± 0.08	0.27 ± 0.01	40.63 ± 1.31
A1	3.31 ± 0.08	0.13 ± 0.01	0.123 ± 0.003	29.29 ± 0.38
O1	1.348 ± 0.031	1.965 ± 0.110	0.218 ± 0.012	26.93 ± 0.76
Flow to A2	3.93 ± 0.11	1.83 ± 0.05	0.242 ± 0.007	38.04 ± 1.05
A2	3.92 ± 0.05	0.162 ± 0.008	0.157 ± 0.004	26.21 ± 0.54
O2	1.539 ± 0.010	2.115 ± 0.033	0.331 ± 0.003	25.33 ± 0.22
Flow to A3	3.95 ± 0.09	1.98 ± 0.06	0.353 ± 0.010	35.83 ± 0.63
A3	3.84 ± 0.13	0.303 ± 0.015	0.192 ± 0.005	26.21 ± 0.35
O3	1.64 ± 0.03	2.458 ± 0.134	0.294 ± 0.008	26.13 ± 0.51
Flow to A4	3.89 ± 0.18	2.31 ± 0.07	0.311 ± 0.015	35.94 ± 1.18
A4	3.77 ± 0.07	0.139 ± 0.004	0.067 ± 0.002	26.24 ± 0.94
O4	1.15 ± 0.09	2.481 ± 0.171	0.281 ± 0.009	25.64 ± 1.15
Flow to A5	3.31 ± 0.04	2.34 ± 0.08	0.32 ± 0.01	34.92 ± 0.46
A5	3.15 ± 0.14	0.137 ± 0.004	0.007 ± 0.0002	26.90 ± 0.67
O5	1.025 ± 0.060	2.02 ± 0.13	0.126 ± 0.004	24.24 ± 0.29
MBR	0.63 ± 0.01	2.443 ± 0.090	0.172 ± 0.006	20.17 ± 0.73

[a] The ammonia nitrogen and COD concentrations of influent on Day 242 were 41.27 and 202.15 mg/L respectively, which was different from the data of flow to A1 due to the multiple step-feed strategy and the dilution by returned activated sludge.

spatial fashions had been reported to be favourable for AOB against NOB (Xu et al., 2017; Zeng et al., 2010; Gu et al., 2017; Kornaros et al., 2008; Yang and Yang, 2011), this study further showed that the combined control of DO, SRT and sludge return ratio was critical towards a stable nitrite shunt in a continuous step-feed biological system as illustrated in Fig. 1.

In addition, the COD removal in each chamber of the system was also shown in Table 2. The results clearly confirmed that most of COD was removed through denitritation in the anoxic chambers. On the other hand, as less COD was aerobically oxidized, the aeration-associated energy consumption for COD oxidation could be reduced substantially compared to the conventional activated sludge process in which about 50% of the total energy was utilized for aeration (Henderson, 2002).

3.3. Batch experiments and microbial abundances associated with nitrogen removal

In this study, the laboratory batch experiments were conducted with municipal wastewater and activated sludge both taken from the pilot plant with the aim to validate nitrite shunt observed in the pilot plant. Table 3 showed the concentration profiles of nitrogen compounds in the batch experiment from which the specific nitrite and nitrate production rates were determined to be 12.63 mg·(g VSS h)$^{-1}$, and 0.75 mg·(g VSS h)$^{-1}$, respectively, i.e. the specific AOB activity was about 15.8 times higher than that of NOB. As the result, a high NAR of 96% was achieved, which was in agreement with the NAR obtained in the pilot plant.

Fig. 4 further showed a significant decrease in the NOB abundance including *Nitrospira* and *Nitrobacter* in Phase III during the operation of the pilot plant (i.e. the period of the DO concentration in the MBR reduced from 2.5 mg/L to 1.5 mg/L), which was associated with an increase of the NAR from below 20% to about 80% (Fig. 3 (b)) due to the successful suppression of NOB in the MBR and the oxic chambers of the biological tank. Compared to NOB, the abundance of AOB remained relatively stable and gradually became

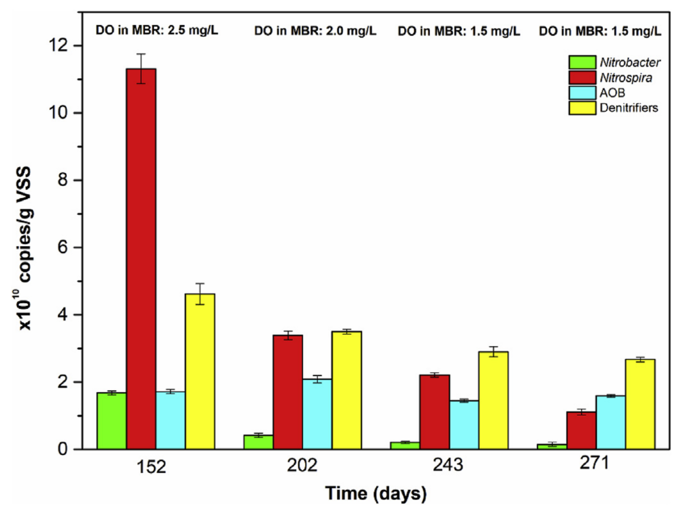

Fig. 4. Abundances of 16S rRNA genes of key functional species associated with nitrogen removal.

dominant against NOB (Fig. 4). These in turn provided microbiological evidence showing the stable nitritation-denitritation achieved in the pilot plant. In addition, *Nitrospira* was found to be the dominant NOB over *Nitrobacter* in the pilot plant. In fact, it had been reported that the relative abundances of *Nitrospira* and *Nitrobacter* was essentially determined by some environmental factors, e.g. low DO concentration (i.e. DO < 1.0 mg/L), short SRT (i.e. 4.27 days) and high temperature (i.e. 29–30 °C) favoured the growth of *Nitrospira* instead of *Nitrobacter* (Huang et al., 2010). These indeed were helpful for explaining the observed dominance of *Nitrospira* over *Nitrobacter* as the pilot plant was operated in the similar conditions.

In addition, a reduction of denitrifiers was obtained in anoxic chambers, which was due to the lower biomass production during denitritation in Phase III compared with denitrification in Phase II.

Table 3
Concentration profiles of nitrogen compounds in the laboratory batch experiments. (mg/L).

Time (min)	0	30	60	90	120
NH_4^+-N	20.31 ± 0.57	14.86 ± 0.35	12.14 ± 0.28	9.87 ± 0.08	7.33 ± 0.19
NO_2^--N	2.13 ± 0.09	4.96 ± 0.13	7.40 ± 0.18	9.84 ± 0.05	12.11 ± 0.14
NO_3^--N	0.15 ± 0.03	0.33 ± 0.01	0.41 ± 0.06	0.44 ± 0.03	0.46 ± 0.04

Therefore, these results further confirmed the observations in the pilot plant that NOB was successfully suppressed by the proposed operation strategy through the adjustment of aeration intensity in MBR in combination with the holistic control of DO, SRT and sludge return ratio.

4. Conclusions

It was demonstrated probably for the first time that stable mainstream nitritation-denitritation was achievable in a pilot step-feed plant of 30 m^3/day coupled with MBR for treating low strength municipal wastewater. The system was successfully stabilized by adjusting aeration intensity in MBR together with the integrated control of DO, SRT and sludge return ratio. By adopting such operation strategy, both the batch experiments and microbial analysis clearly showed that NOB was successfully suppressed in the pilot plant, e.g. the abundance and activity of AOB were 1.7 and 16.8 times of NOB. Meanwhile, about 90% of COD and NH_4^+-N were removed via nitritation-denitritation without the addition of external organic carbon source in the pilot plant. Moreover, high quality permeate could be produced from the integrated pilot plant. Consequently, it appears highly feasible to realize stable mainstream nitritation-denitritation in the process configuration explored in this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2018.10.187.

References

Araki, N., Yamaguchi, T., Yamazaki, S., Harada, H., 2004. Quantification of amoA gene abundance and their amoA mRNA levels in activated sludge by real-time PCR. Water Sci. Technol. 50, 1–8.

Aslan, S., Simsek, E., 2017. Optimization of operational conditions for nitrite accumulation in a submerged biofilter. Environ. Eng. Manag. J. 16 (10), 2267–2274.

AWWA, A., 1998. WEF Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington DC, USA.

Cai, W., Liu, J., Zhang, X., Ng, W.J., Liu, Y., 2016. Generation of dissolved organic matter and byproducts from activated sludge during contact with sodium hypochlorite and its implications to on-line chemical cleaning in MBR. Water Res. 104, 44–52.

Dionisi, H.M., Layton, A.C., Harms, G., Gregory, I.R., Robinson, K.G., Sayler, G.S., 2002. Quantification of Nitrosomonas oligotropha-like ammonia-oxidizing bacteria and Nitrospira spp. from full-scale wastewater treatment plants by competitive PCR. Appl. Environ. Microbiol. 68, 245–253.

Degrange, V., Bardin, R., 1995. Detection and counting of Nitrobacter populations in soil by PCR. Appl. Environ. Microbiol. 61, 2093–2098.

Eaton, A., Clesceri, L., Greenberg, A., Franson, M., 1998. Standard Methods for Examination of Water and Waste Water, twentieth ed. American Public Health Association, Washington, DC.

Erdirencelebi, D., Koyuncu, S., 2017. Operational strategies and environmental conditions inducing aerobic denitritation in short-cut biological nitrogen removal at side-line treatment. J. Environ. Sci. Health Part A Toxic/hazardous Substances Environ. Eng. 1.

Ge, S., Peng, Y., Qiu, S., Zhu, A., Ren, N., 2014. Complete nitrogen removal from municipal wastewater via partial nitrification by appropriately alternating anoxic/aerobic conditions in a continuous plug-flow step feed process. Water Res. 55, 95–105.

Gu, J., Xu, G., Liu, Y., 2017. An integrated AMBBR and IFAS-SBR process for municipal wastewater treatment towards enhanced energy recovery, reduced energy consumption and sludge production. Water Res. 110, 262–269.

Guisasola, A., Jubany, I., Baeza, J.A., Carrera, J., Lafuente, J., 2005. Respirometric estimation of the oxygen affinity constants for biological ammonium and nitrite oxidation. J. Chem. Technol. Biotechnol. 80, 388–396.

Henderson, M., 2002. Energy Reduction Methods in the Aeration Process at Perth Wastewater Treatment Plant. University of Strathclyde. Renewable Energy Systems and the Environment MSc Individual Theses, 2001-2002.

Hou, J., Xia, L., Ma, T., Zhang, Y., Zhou, Y., He, X., 2017. Achieving short-cut nitrification and denitrification in modified intermittently aerated constructed wetland. Bioresour. Technol. 232, 10.

Huang, Z., Gedalanga, P.B., Asvapathanagul, P., Olson, B.H., 2010. Influence of physicochemical and operational parameters on Nitrobacter and Nitrospira communities in an aerobic activated sludge bioreactor. Water Res. 44, 4351–4358.

Hulle, S.W.H.V., Vandeweyer, H.J.P., Meesschaert, B.D., Vanrolleghem, P.A., Dejans, P., Dumoulin, A., 2010. Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams. Chem. Eng. J. 162, 1–20.

Kornaros, M., Marazioti, C., Lyberatos, G., 2008. A pilot scale study of a sequencing batch reactor treating municipal wastewater operated via the UP-PND process. Water Sci. Technol. 58, 435–438.

Laureni, M., Falås, P., Robin, O., Wick, A., Weissbrodt, D.G., Nielsen, J.L., Ternes, T.A., Morgenroth, E., Joss, A., 2016. Mainstream partial nitritation and anammox: long-term process stability and effluent quality at low temperatures. Water Res. 101, 628–639.

Lee, H., Tan, T.P., 2016. Singapore's experience with reclaimed water: newater. Int. J. Water Resour. Dev. 32 (4), 1–11.

Lemaire, R., Marcelino, M., Yuan, Z., 2008. Achieving the nitrite pathway using aeration phase length control and step-feed in an sbr removing nutrients from abattoir wastewater. Biotechnol. Bioeng. 100, 1228.

Regmi, P., Miller, M.W., Holgate, B., Bunce, R., Park, H., Chandran, K., Wett, B., Murthy, S., Bott, C.B., 2014. Control of aeration, aerobic SRT and COD input for mainstream nitritation/denitritation. Water Res. 57, 162–171.

Regmi, P., Bunce, R., Miller, M.W., Park, H., Chandran, K., Wett, B., Murthy, S., Bott, C.B., 2015. Ammonia-based intermittent aeration control optimized for efficient nitrogen removal. Biotechnol. Bioeng. 112, 2060–2067.

Sliekers, A.O., Haaijer, S.C., Stafsnes, M.H., Kuenen, J.G., Jetten, M.S., 2005. Competition and coexistence of aerobic ammonium- and nitrite-oxidizing bacteria at low oxygen concentrations. Appl. Microbiol. Biotechnol. 68 (6), 808–817.

Strous, M., Heijnen, J., Kuenen, J.G., Jetten, M., 1998. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. Appl. Microbiol. Biotechnol. 50 (5), 589–596.

Throbäck, I.N., Enwall, K., Jarvis, Å., Hallin, S., 2004. Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE. FEMS Microbiol. Ecol. 49, 401–417.

Vadivelu, V.M., Yuan, Z., Fux, C., Keller, J., 2006. The inhibitory effects of free nitrous acid on the energy generation and growth processes of an enriched nitrobacter culture. Environ. Sci. Technol. 40, 4442–4448.

Wu, J., He, C., van Loosdrecht, M.C., Pérez, J., 2016. Selection of ammonium oxidizing bacteria (AOB) over nitrite oxidizing bacteria (NOB) based on conversion rates. Chem. Eng. J. 304, 953–961.

Xu, G., Wang, H., Gu, J., Shen, N., Qiu, Z., Zhou, Y., Liu, Y., 2017. A novel AB process for enhanced biological nutrient removal in municipal wastewater reclamation. Chemosphere 189, 39.

Xu, G., Zhou, Y., Yang, Q., Lee, Z.M.-P., Gu, J., Lay, W., Cao, Y., Liu, Y., 2015. The challenges of mainstream deammonification process for municipal used water treatment. Appl. Microbiol. Biotechnol. 99 (6), 2485–2490.

Yang, S., Yang, F., 2011. Nitrogen removal via short-cut simultaneous nitrification and denitrification in an intermittently aerated moving bed membrane bioreactor. J. Hazard Mater. 195, 318–323.

Zeng, W., Li, L., Yang, Y., Wang, S., Peng, Y., 2010. Nitritation and denitritation of domestic wastewater using a continuous anaerobic–anoxic–aerobic (A 2 O) process at ambient temperatures. Bioresour. Technol. 101, 8074–8082.